Remodeling of the Actin Cytoskeleton and Contraction in the A7r5 Smooth Muscle Cell.

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Abstract

Data suggest that differential remodeling of alpha-actin and beta-actin could play a necessary role in smooth muscle's unique contractile properties. When smooth muscle cells are stimulated by Phorbol ester (PDBu), the actin in the cells appears to remodel into podosomes, which are putatively important structures in smooth muscle contraction. Data suggest that the formation of these podosomes primarily involves the remodeling of alpha-actin, while beta-actin remodeling is hypothesized to function in holding the contracting smooth muscle cells in their shortened configuration. The effect of rho-kinase (ROCK) inhibition on the structure of actin in smooth muscle was examined by time-course treatment of A7r5 smooth muscle cells with the ROCK inhibitor Y-27632 and subsequent differential immunofluorescent staining of alpha-actin and beta-actin. The data suggest that ROCK inhibition leads to the dissolution of extant alpha-actin stress cables. Furthermore, the data suggest that the beta-actin ultrastructure in smooth muscle cells is less affected by ROCK inhibition. These data support the existence of a differential mechanism for the regulation of alpha-actin and beta-actin maintenance and remodeling in smooth muscle cells

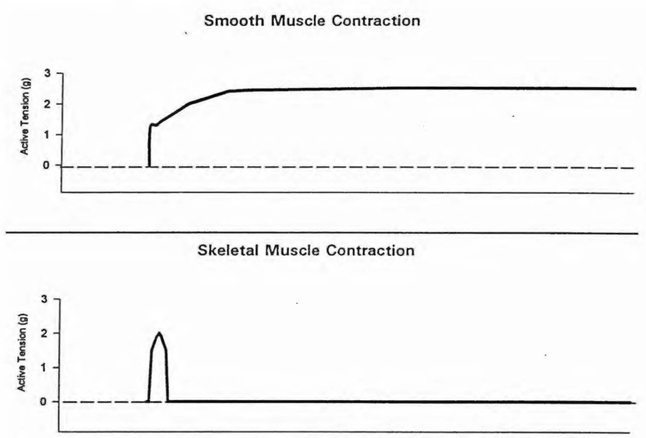

Figure 1. Model comparing development of tension in smooth (top) and skeletal (bottom) muscle. Smooth muscle is able to generate as much tension as skeletal muscle. However, smooth muscle may contain only 20% of the myosin that is found in skeletal muscle. Also smooth muscle is able to maintain contraction with only 0.35% of the energy consumption of skeletal muscle.

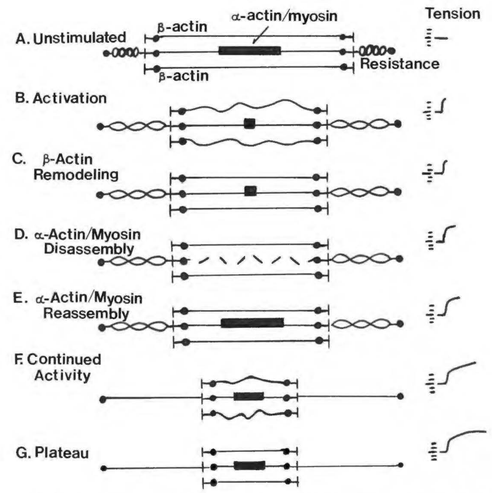

Figure 2. Model explaining force development and maintenance of active tension in smooth muscle. *

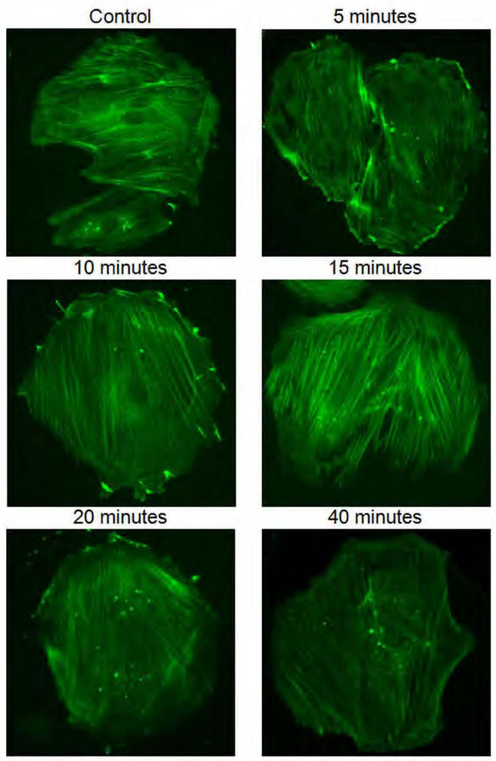

Figure 3. Micrographs demonstrating the effect of 1 μM Y-27632 on beta-actin structure in resting A7r5 smooth muscle cells over a time course of 40 minutes

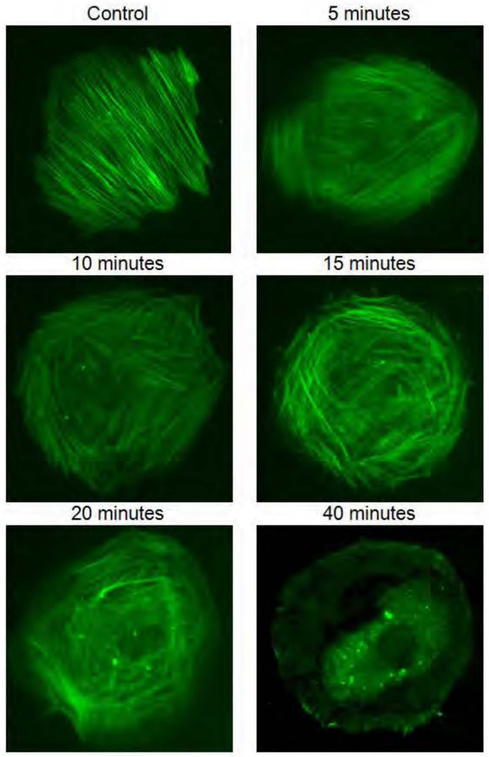

Figure 4. Micrographs demonstrating the effect of 1μM Y-27632 on alpha-actin structure in resting A7r5 smooth muscle cells over a time course of 40 minutes

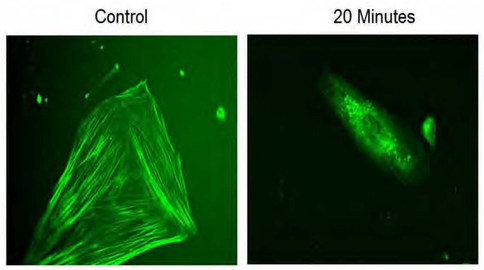

Figure 5: Micrograph comparing alpha-actin structure in a control cell (top left) and a cell that was treated with 10^{-8}M PDBu for 20 minutes (top right) indicating remodeling of the a-actin cytoskeleton and podosome formation

Conclusions

•Inhibition of Rho-kinase in resting cells induces a dissolution of the alpha-actin ultrastructure.

•Rho-kinase may play a role in maintaining the α-actin ultrastructure in the resting A7r5 smooth muscle cell.

•Rho-kinase inhibition does not appear to have a drastic effect on resting beta-actin filament structure.

•Beta-actin does not appear to be as susceptible to ROCK-Inhibition-mediated fiber dissolution as alpha-actin.

•This suggests that there may be separate biochemical pathways regulating the alpha-actin and beta-actin filament structure.

Future Experiments

•Examine the effects of PDBu stimulation on the levels of G and F actin in smooth muscle cells

•Use GFP hybridization of alpha actin to observe actin remodeling in real time

•Use RNA interference to further confirm differential regulation of alpha and beta-actin ultrastructure

Acknowledgements

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•*Ca2+-dependent actin remodeling in the contracting A7r5 Cell, C. LI, M.E. Fultz, J. Parkash, W.B. Rhoten asd G.L. Wright, *Muscle Research and Cell Motility*, 22: 521-534, 2001